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16. ABSTRACT
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Mutagenic specificity and the prediction of mechanisms and bioactivation pathways of genotoxicants: the mutagenicity of 5-nitroacenaphthene as an example

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Summary

5-Nitroacenaphthene induces primarily mutations of the frameshift type in *Salmonella typhimurium* (i.e., strains TA98 and TA1538). Addition of activation mixtures (S9) results in an increased mutagenic potency as well as a shift in specificity to base-substitution mutations (i.e., strain TA100). The magnitude of these effects depends upon the species of origin of the S9 and whether the animals were induced (Aroclor 1254). These effects are ascribed to the S9-mediated conversion of 5-nitroacenaphthene to additional mutagenic metabolites (possibly ring oxidation products).

In addition, while 5-nitroacenaphthene exhibited appreciable activity in the absence of S9 for strain TA98, its mutagenicity for TA97, also an indicator of frameshifts, was negligible. TA97 differs from TA98 in the nucleotide sequence at the mutational hot spots (a run of 6 cytosine as opposed to 8 alternating guanine–cytosine residues).

These findings indicate that a careful analysis of the results obtained in the standard *Salmonella typhimurium* mutagenicity assay may yield useful information regarding bioconversion pathways and the role of DNA sequence in the ultimate expression of pro-mutational events.

Because of the current concern of the potential health hazards that could result from the presence of a plethora of nitrated polycyclic aromatic hydrocarbons (nitroarenes) in the environment, considerable emphasis has been placed on the

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study of their genetic and genotoxic properties (Rosenkranz and Mermelstein, 1982). Also, because an estimation of their potential risk depends upon a detailed knowledge of the various potential pathways for detoxification, a study of their metabolism is deemed to be appropriate. A number of model nitroarenes have recently been investigated and this has resulted in the realization that there existed a diversity of bioactivation pathways which involved either reduction of the nitro function or oxidation of the aromatic moiety or a combination of these (Rosenkranz and Mermelstein, 1982; Rosenkranz et al., 1982). Thus, in an earlier study (McCoy et al., 1983) we focused on the mutagenic properties of 5-nitroacenaphthene (5-NAN, 1,2-dihydro-5-nitroacenaphthylene, CASR No. 602-87-9). We were able to infer from the results that in bacteria (*Salmonella typhimurium*), mutagenicity was dependent upon reduction of the nitro function to the corresponding arylhydroxylamine which was followed by esterification of the latter to the hydroxamic acid ester. However, by using microbial indicator strains blocked either in their ability to reduce the nitro group or to esterify the hydroxylamine, we were able to demonstrate that post-microchondrial activation mixtures (e.g., S9) derived from the livers of Aroclor-induced rats, were able to by-pass these blocks, presumably as the result of ring oxidation in accordance with the scheme described by El-Bayoumy and Hecht (1982).

The present study was undertaken to determine whether these dichotomies in bioactivation schemes could have been predicted from an analysis of the data generated from standard Salmonella mutagenicity assays. If this could be accomplished, it would have the advantage of permitting a prediction of the various bioactivation schemes based upon the simple, rapid and standardized procedures, and such results could then be used as guides for planning of the more complex, time-consuming metabolic studies. We report herein that indeed, an analysis of the mutagenic specificities indicate that the intermediate(s) responsible for the mutagenic activities in the presence and absence of S9 differ and moreover that there are differences in the catalytic properties of S9 preparations which depend upon induction of cytochrome P450 monooxygenases and species of origin.

Materials and methods

5-NAN, the same preparation that was used previously (McCoy et al., 1983), was obtained from the National Cancer Institute Chemical Repository at IIT Research Insitute, Chicago (IL). It originated from the same batch that was used for the National Cancer Institute Bioassay Program (Dunkel and Simmon, 1980; Griesemer and Cuoto, 1980). Its purity has been reported as 99% based upon HPLC behavior. Mutagen standards (AF2, sodium azide, 2-nitrofluorene, 9-aminoacridine and 2-aminoanthracene) were also received from the National Cancer Institute Chemical Repository.

The Salmonella mutagenicity assay was carried out as described by Ames and associates (1975) using cultures growing exponentially in trypticase soy broth. Microsomes (S9) were prepared as described by Ames et al. (1975) from the livers of

TABLE I

MUTAGENICITY OF 5-NITROACENAPHTHENE

S9	µg/plate	Revertants per plate						
		TA1535	TA100	TA1537	TA1538	TA98	TA97	WP2uvrA
None	0	10	86	5	5	7	75	18
	0.33	11	83	9	4	12	89	13
	1.0	10	114	7	7	22	81	18
	3.3	13	133	5	13	28	75	15
	10	12	159	17	28	144	96	19
	33	24	442	19	131	411	122	17
	100	52	880	56				21
	333							48
	Rev./µg	0.43	9.8	0.48	3.9	12.4	1.4	0.10
RN	0	6	71	5	8	12		18
	0.33	8	82	4	16	13		16
	1.0	9	95	12	23	19		15
	3.3	6	174	12	48	34		21
	10	6	336	28	136	82		16
	33	12	920	17	157	125		21
	100	22	110	7	168	36		24
	333	20						57
	Rev./µg	0.16	25.6	2.3	12.6	7.0		0.12
RI	0	6	103	8	8	11		17
	0.33	8	131	9	25	20		14
	1.0	8	166	14	43	22		13
	3.3	20	349	32	192	157		22
	10	37	808	70	314	329		23
	33	63	1665	87	816	734		50
	100		1858	16	399	650		59
	333							77
	Rev./µg	1.6	70.6	6.2	30.7	21.5		0.96
MN	0	8	153	9	8	17		28
	0.33	6	141	12	9	31		28
	1.0	11	157	13	13	42		31
	3.3	13	253	19	24	124		40
	10	21	556	27	41	302		38
	33	20	638	25	118	559		43
	100	40	1213			5		39
	333							
	Rev./µg	1.3	41.0	1.7	3.3	28.5		0.88
MI	0	11	148	9	7	18		26
	0.33	16	138	13	6	21		23
	1.0	13	209	9	13	32		26
	3.3	20	263	31	24	106		23
	10	24	729	53	101	276		31
	33	51	2022	43	461	984		64
	100	68	1110			1027		65
	333							64
	Rev./µg	1.1	57.3	4.2	14.1	30.7		1.1

TABLE I (continued)

S9	μg/plate	Revertants per plate						
		TA1535	TA100	TA1537	TA1538	TA98	TA97	WP2uvrA
HN	0	9	90	5	8	10	152	21
	0.33	8	161	9	28	35	178	19
	1.0	9	333	8	64	102	198	22
	3.3	13	684	44	171	346	324	25
	10	75	1462	83	277	668	471	38
	33	49	1729	40	41	663	347	43
	100		1104	58				135
	333							71
	Rev./μg	1.1	177.0	7.9	48.8	102.4	31.3	1.1
HI	0	9	88	7	11	8		18
	0.33	9	184	4	23	46		19
	1.0	15	205	21	79	131		19
	3.3	25	1011	55	364	501		30
	10	58	1265	148	1008	955		23
	33	23	1890	133	865	648		127
	100		1193			8		55
	333							91
	Rev./μg	4.9	283.1	14.1	101.5	151.0		0.41

N, uninduced; I, Aroclor 1254-induced.

R, rat; M, mouse; H, hamster.

Protein contents of the S9 preparations were: RN 38.7 ± 5.6; RI 42.8 ± 6.3; MN 38.6 ± 7.9; MI 40.8 ± 3.4; MI 40.8 ± 3.4; HN 36.7 ± 9.4; HI 40.8 ± 6.0 mg per ml.

normal or Aroclor 1254-induced animals: rats (Fisher 344), hamsters (Syrian; Jackson Laboratories), or mice B6C3F1 (C57B16 × C3H(He)). The protein contents of the S9 preparations are given in the legend to Table I.

Salmonella typhimurium tester strains TA1535, TA1537, TA1538, TA97, TA98 and TA100 were kindly provided by Dr. Bruce N. Ames, University of California, Berkeley (Ames et al., 1975; Levin et al., 1982).

The mutagenicity of test chemicals for *E. coli* WP2*uvrA* was determined according to the procedure of Green and Muriel (1976). This strain responds primarily to base substitution mutagens (reversions from *ochre* mutation or its suppression).

Each chemical was assayed on at least two separate occasions and each dose level was tested in triplicate. Mutagenic activity (revertants per μg) was calculated from the linear (ascending) portion of the dose–response curves.

Results and discussion

In the absence of S9, 5-NAN exhibited primarily mutagenicity for Salmonella strains TA1538 and TA98, i.e., the strains usually used to detect frameshift muta-

tions (Table 1). There is little activity toward Salmonella strain TA1535 and for *E. coli* WP2*uvrA*, the 2 strains that are used to measure base-substitution mutations. Salmonella strain TA100 is a plasmid-containing derivative of TA1535, however introduction of the plasmid has been shown to result in a loss of mutagenic specificity. With respect to structural analogs of 5-NAN, the present results are in agreement with the earlier findings that while nitronaphthalenes are endowed with base-substitution acitivity, addition of a third ring structure (e.g., naphthalic acid imides) resulted in the pattern characteristic of frameshift activity (McCoy et al., 1981). Again, as with many other nitro containing chemicals, activity in strain TA1535, was enhanced by the presence of plasmid pKM101 (e.g., TA100) (Rosenkranz and Mermelstein, 1982).

It should be noted that while 5-NAN exhibited appreciable activity for the standard frameshift indicator strains, i.e., TA1538 and TA98, it showed only negligible activity towards strain TA97, a newly constructed tester strain which has also been reported to detect frameshift mutations (Levin et al., 1982). This anomaly is discussed below.

As reported earlier (McCoy et al., 1983), in contrast to the behavior of other nitroarenes, the addition of S9 resulted in a significant increase in the mutagenic activity of 5-NAN, thus confirming our earlier results with this chemical. In the present report this was extended further to inclued S9 preparations derived from the livers of mice, hamsters, and rats, both uninduced as well as induced with Aroclor 1254. The extent of this augmentation was dependent upon the species of origin of the S9 and for the most part was more elevated when it was derived from the livers of Aroclor-induced animals. These effects can be illustrated more strikingly when mutagenic activities are normalized to those obtained with S9 derived from the livers of Aroclor-induced rats (Table 2).

It is also of significance that while in the absence of S9 the mutagenicity of 5-NAN was highest in strain TA98 (i.e., indicative of frameshift mutation), in the presence of S9 the mutagenicity was consistently higher in strain TA100 as well as becoming significant for *E. coli* WP2*uvrA* and TA1535 (see analysis in Table 3). Thus suggesting that the increased mutagenicity mediated by S9 is not simply due to a cooperative action between bacterial and mammalian enzymes (nitroreductases and esterification enzymes) but rather to a different type of mutagenic intermediate which induces base substitution mutations and which possibly is not dependent upon the reduction of the nitro function to the corresponding arylhydroxylamine and its subsequent esterification. This would be in line with the earlier results using nitroreductase and esterification-deficient tester strains (McCoy et al., 1983) and suggests a role of ring oxidation products (El-Bayoumy and Hecht, 1982).

It is also of significance that, in the absence of S9, 5-NAN exhibited only marginal activity for strain TA97, a recently constructed plasmid-containing strain that detects frameshift mutagens and that is to replace TA1537 (Levin et al., 1982). However, the mutational hot-spot of TA97 differs from that of TA98 (and TA1538), i.e., a run of 6 cytosine and 8 alternating guanine–cytosine residues, respectively (Ames et al., 1975; Isono and Yourno, 1974; Levin et al., 1982; Maron and Ames, 1983). These results indicate not only that there is a shift in the mutagenic specificity

TABLE 2
RELATIVE EFFECTS OF ACTIVATION PREPARATIONS

S9	TA1535	TA100	TA1537	TA1538	TA98	WP2uvrA
None	0.27	0.14	0.08	0.13	0.58	0.10
RN	0.10	0.36	0.37	0.41	0.33	0.13
RI	1.00	1.00	1.00	1.00	1.00	1.00
MN	0.81	0.58	0.27	0.11	1.33	0.92
MI	0.69	0.81	0.68	0.46	1.43	1.15
HN	0.69	2.51	1.27	1.59	4.76	1.15
HI	3.06	4.01	2.27	3.31	7.02	0.43

Mutagenicities for each strain were normalized to the activities exhibited in the presence of RI.
N, uninduced; I, Aroclor 1254-induced.
R, rat; M, mouse; H, hamster.

of 5-NAN in the presence of S9, thus further confirming the earlier results, but more importantly that the base sequence of the target DNA may also be of importance in the final outcome of the pro-mutational event. Although, the exact nature of this effect of DNA sequence is as yet incompletely understood, it might be hypothesized that the fundamental difference between the 2 target sequences is the fact that in TA98 we are dealing with an alternating guanine–cytosine sequence (Heller et al., 1983). It is known that such a sequence is a prerequisite for the formation of the left-handed Z DNA conformation (Wang et al., 1979). It may then be hypothesized further that the DNA adduct formed by 5-NAN reacts with the guanine moiety (e.g., to form *N*-(deoxyguanosin-8-yl)-5-aminoacenaphthene) and causes it to shift from the usual *anti* to the *syn* configuration which is a requirement for the formation of the Z conformation. Moreover, it has been shown further that the formation of the Z DNA in a short segment of an alternative guanine–cytosine sequence in the middle of a DNA molecule results in profound alterations of the supercoil properties of DNA (Klysik et al., 1982; Stirdivant et al., 1982; Zacharias et al., 1982). This possibly extends the genetic effect beyond the immediate vicinity of the original

TABLE 3
COMPARATIVE MUTAGENIC SPECIFICITIES

S9	TA1535	TA100	TA1537	TA1538	TA98	TA97	WP2uvrA
None	0.04	0.79	0.04	0.32	1.00	0.12	0.01
RN	0.02	3.66	0.33	1.80	1.00		0.02
RI	0.07	3.28	0.29	1.43	1.00		0.05
MN	0.05	1.44	0.06	0.12	1.00		0.03
MI	0.04	1.87	0.14	0.46	1.00		0.04
HN	0.01	1.73	0.08	0.48	1.00	0.31	0.01
HI	0.03	1.88	0.09	0.67	1.00		0.00

Relative mutagenicities for each S9 were normalized to the activities exhibited in strain TA98.
N, uninduced; I, Aroclor 1254-induced.
R, rat; M, mouse; H, hamster.

DNA sequence. This, in turn, might explain the appreciable mutagenicity of 5-NAN towards TA98 and minimal effect in TA97 in the absence of S9. Certainly, this mechanistic possibility deserves further investigation as it might be essential to an understanding of mutagenic events in general.

In conclusion, therefore, the present investigation of the properties of 5-NAN in a battery of standard Salmonella tester strains, both in the presence as well as in the absence of S9, suggests that this chemical is capable of a multiplicity of mutagenic and genotoxic activities and this information, in turn, can be used to design experiments to identify and clarify these. Moreover, the present results indicate the usefulness of a careful examination of mutagenic responses obtained in the Salmonella assay in the absence and presence of S9 as unexpected or atypical results may indicate unusual bioactivation/detoxification pathways.

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